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Etching kinetics of a self-etching primer ☆

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Abstract

Self-etching primers are thought to offer significant advantages over total-etch adhesive systems. The hypothesis tested in this study was that there was no difference in etching characteristics between a self-etching primer and a phosphoric acid solution at the same pH. Etching was assessed using atomic force microscopy (AFM) evaluation of site-specific changes in the height of the peritubular and intertubular dentin as a function of exposure time.

Human dentin disks ($n = 6$/group), prepared with an acid-resistant glass reference layer, were etched with a self-etching primer and with 0.0134 M phosphoric acid (both $\text{pH} \cong 1.94$). Depth changes relative to the reference layer were measured with the AFM after each etching interval, at 15 different locations, each in the peritubular and intertubular dentin. The total demineralization depth was measured in a scanning electron microscope. Peritubular dentin etching rate was linear while it could be measured (up to 15 s) and was greater for the self-etching primer ($p < 0.0001$). Intertubular dentin displayed a similar demineralization pattern with both acids, ultimately reaching a plateau in the majority of specimens. The self-etching primer attained a plateau after less recession than phosphoric acid ($p < 0.0001$). Dentin demineralization appears to be affected by other factors in addition to the pH of the etchant solutions. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Self-etching primer; AFM; Demineralization; Phosphoric acid

1. Introduction

Current adhesive dentistry relies primarily on the total etch technique [1] to promote mechanical interlocking of a resin-based material with the demineralized tooth structure [2]. Bonding to dentin, however, is more challenging than to enamel, mostly because of the complex hydrated structure of dentin [3]. Hydrophilic primers are often used to facilitate the penetration of the monomer of the bonding resin into the demineralized dentin [4,5], with the creation of a hybrid layer [2]. These recently improved dentin bonding systems, still show some technique sensitivity, and even the quantity of moisture on the dentin substrate affects their adhesion [6].

The introduction of self-etching primers that combine the tooth conditioning and priming steps, and all-in-one adhesive systems that bring together the three adhesive steps, seems to be a promising approach for reducing technique sensitivity [7]. These materials may overcome some of the shortcomings of the earlier adhesive systems [8,9], such as poor impregnation of the demineralized dentin layer [10]. Theoretically, this should give higher long-term bond strength than the non-self-etching systems [11]. On the other hand, since the acidic monomer is not rinsed away, it has to be buffered by the tooth structure or immobilized by polymerization and there has been some concern that the demineralization extends beyond the depth of monomer infiltration. Since these materials present a fairly novel concept, studies on their demineralization characteristics are needed.

Several studies undertaken in our laboratory [12–17] have demonstrated the potential of the atomic force microscope (AFM) for the direct observation of tooth demineralization since it combines high resolution (vertical resolution of 0.1 nm and in-plane resolution of ~20 nm) with the ability to operate in solution, avoiding the desiccation of dentin [18]. Those studies have successfully established a protocol for AFM measurements of the dimensional and microstructure

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changes in dentin subjected to a variety of different treatments (dehydration, rehydration, demineralization). Nevertheless, a stable reference layer resistant to both acid and solvent is necessary [15]. AFM measurements of surface recession tend to have an accuracy of about 10 nm. Scanning electron microscope (SEM) measurements of demineralization depth are less accurate not only because of specimen variability, and roughness, but also because of slight shrinkage of demineralized dentin during processing for SEM. This limits its accuracy to about 0.5 μm.

In this work, we developed a glass reference layer method and used an AFM to study the effect of a self-etching primer (pH ∼ 1.94) (Clearfil SE Bond, Kuraray America, New York, NY) on the etching characteristics and kinetics of peritubular and intertubular dentin, and compared these effects to that of a diluted phosphoric acid solution (pH ∼ 1.94) to test the null hypothesis that the self-etching primer and phosphoric acid at the same pH show the same demineralization characteristics.

3. Materials and methods

3.1. Specimen preparation

The specimens used in this study were prepared from human non-carious third molars. All the teeth were recently extracted from patients needing extractions as a part of their dental treatment, as approved by the UCSF Committee on Human Research. Teeth were gamma irradiated [19] and refrigerated at 4°C in Hank's balanced salt solution (HBSS) prior to use.

Fig. 1 shows a schematic of the specimen preparation and study procedures. Six teeth were sectioned parallel to the occlusal surface with a low-speed diamond saw (Buehler-Lake Bluff, IL) to remove an occlusal dentin disk, 2 mm thick, that was then sectioned in half. All disks were obtained from approximately the same tooth depth. To create an acid-resistant glass reference layer for height measurements, each sectioned surface of the half disk was polished through 0.05 μm alumina powder slurry (Buehler Micropolish, Buehler Ltd., Lake Bluff, IL). This surface was bonded to sandblasted (12 μm aluminum oxide powder, Buehler-Lake Bluff, IL) 0.5 μm × 0.5 μm glass squares (Microscope cover glass, No. 1 thinness, Rochester Scientific Co., Rochester, NY) that were then acid etched for 5 min with hydrofluoric acid (9.5% hydrofluoric acid, Porcelain etch, Ultradent Products, South Jordan, UT) and silanated (Silane, Ultradent Products, South Jordan, UT). Adhesion between glass and dentin was achieved with a dentin adhesive (Clearfil SE Bond—Kuraray America, New York, NY) and a fluid resin (Fortify, Bisco Inc., Schaumburg, IL).

The specimens were then embedded in plastic material (Coe tray plastic, GC America Inc, Chicago, IL) and polished with six different grits of silicon dioxide paper (Carbimet Buehler-met, Buehler-Lake Bluff, IL) from 240-grit through 1200-grit. The specimen surfaces were polished for 5 min in a polishing machine (Ecomet II—Buehler-Lake Bluff, IL) with Fibrpol PC Discs (1 μm grit—Buehler-Lake Bluff, IL) and ultra-sonically cleaned for 1 min in deionized water. The polishing procedure leaves the dentin surface nearly without a smear layer; therefore, the substrate is not exactly the same as would be encountered clinically.

3.2. AFM examination

The AFM study was performed using contact mode (Nanoscope III, Digital Instruments, Santa Barbara, CA), as described in the previous work [14]. Six specimens were etched with the primer of Clearfil SE Bond (composition—10-MDP, HEMA, hydrophilic dimethacrylate, dl-Camphorquinone, *N*,*N*-diethanol-*p*-

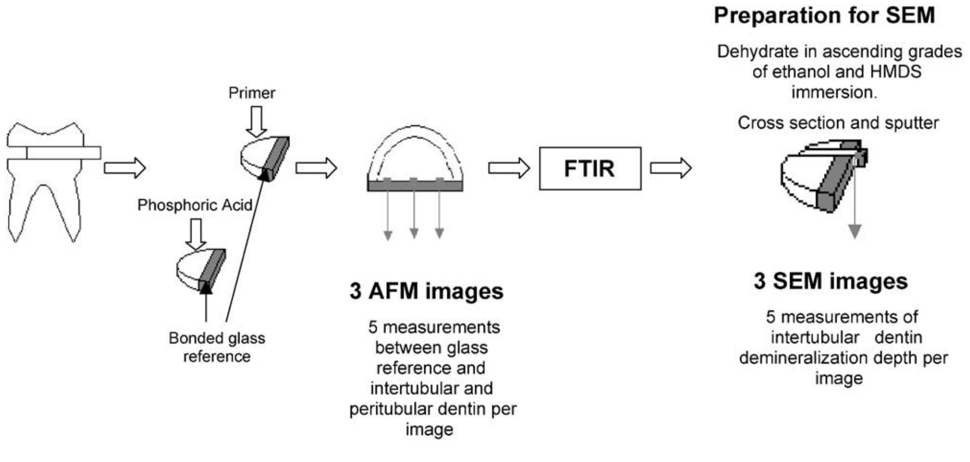


Fig. 1. Schematic of sample preparation and study procedures.

toluidine, water—Batch No. 123A), and the other six with 0.0134 M (0.13%) aqueous phosphoric acid made from 85% phosphoric acid (Fisher Chemical, Fisher Scientific, Fair Lawn, NJ). The pH of both the solutions was measured with an Accumet 1003 handheld pH meter and a glass electrode microprobe (Fisher Scientific, Fair Lawn, NJ) five times, and the mean pH values were 1.94 (±0.008) for both the solutions. For both peritubular and intertubular dentin, depth changes relative to the reference layer were measured under water at five randomly selected locations within each of three 50 μm × 50 μm AFM images. Measurements were taken at baseline (pre-etching) and periodically over a total demineralization time of 35 min. Initial etching periods were shorter (six 5 s periods, followed by 10, 20, and 60 s periods, one each) than later etching periods (3 min, followed by six 5-min periods), in order to identify the time until surface recession stabilized, despite continued application of the etchants, and the recession level at the plateau. After each etching period, specimens were rinsed with ethanol for 5 min to remove the monomer, and soaked in de-ionized water for another 5 min. The 5 min in ethanol were enough to remove the monomer, as confirmed by Fourier Transform Infra-red Spectroscopy (RFX-30 FTIR Spectrometer Laser Precision Analytical, Irvine, CA). The 5 min in de-ionized water were shown, in preliminary studies, to reverse the shrinkage effect of the ethanol.

Depth changes were measured after each etching period between the reference layer and 15 different locations each, for peritubular and intertubular dentin, on each specimen.

3.3. SEM analysis

After AFM examination, three specimens from each group were dehydrated prior to SEM analysis (ISI ABT SX-40A SEM, Topcon Instruments, Pleasanton, CA). They were immersed in 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 12 h at 4°C, and then washed with 20 ml of 0.2 M sodium cacodylate buffer at pH 7.4 for 1 h with three changes of solution. The specimens were rinsed with deionized water for 1 min and dehydrated in ascending grades of ethanol: 25% for 20 min, 50% for 20 min, 75% for 20 min, 95% for 30 min, and 100% for 60 min. They were then dried in hexamethyldisilazane (HMDS, Ted Pella Inc., Redding, CA) for 10 min [20]. This procedure has been extensively studied and proved to be the best process to prepare demineralized dentin specimens for the SEM analysis [21] and minimizes shrinkage. Nevertheless there is always some shrinkage, so the values reported should be regarded as approximate values and not absolute. All SEM specimens in this study were prepared in the same way. Specimens were longitudinally fractured, by applying a shearing force into a precut groove in the specimen base and the cross sections were sputter-coated with 200 nm gold/palladium in a Hummer VII sputtering system (Anatech Ltd, Alexandria, VA). Intertubular dentin demineralization was measured at five different locations of three different images per specimen with image analysis software (Ultrascan 2.1.1, Soft Imaging Software, Kevex Sigma, Noran Instruments, Inc., Madison, WI) while specimens were in the SEM specimen chamber. Three other specimens from each group etched for only 20 s were prepared in the same way for the SEM so that we could estimate the total demineralization depth, for intertubular dentin, at a clinically relevant time.

3.4. Statistical analysis

In intertubular dentin, a demineralization plateau was said to occur if and when a depth change between two etching periods was $<30\%$, and remained $<30\%$ between all subsequent etching periods. We identified the proportion of locations at which demineralization reached a plateau and the depth at the plateau, among those locations with at least 30 min of etching effects measured. The proportions of locations reaching plateaus were estimated and compared across treatment solutions via repeated measures logistic regression models (SAS proc nlmixed [22]). Among locations reaching plateaus, the mean plateau depths were estimated and compared via mixed-effects models (SAS proc mixed [22]).

In peritubular dentin, after 10 s etching the depth was too great to measure at some locations because the AFM tip could not reach the unetched peritubular dentin inside the tubules. In these cases (38% of self-etching primer and 7% of phosphoric acid values), we imputed a depth of 1000 nm, the largest value measured at any peritubular location. The short-term etching rates of each acid for peritubular dentin (10 s) and intertubular dentin (20 s) were estimated and compared using mixed-effects models to account for the nesting of measurement locations within images, and images within disks.

In the SEM data for each etching duration (20 or 2100 s), we compared the conditioners with respect to the mean demineralization depth using mixed-effects models and two-sided 0.05 level tests. These models allowed us to estimate and control for possible correlations among measurements within images and images within disks [22].

4. Results

Fig. 2 shows an AFM sequence of demineralization with the self-etching primer (Fig. 2(a)) and with the phosphoric acid (Fig. 2(b)) at 0, 20, 600 and 2100 s.

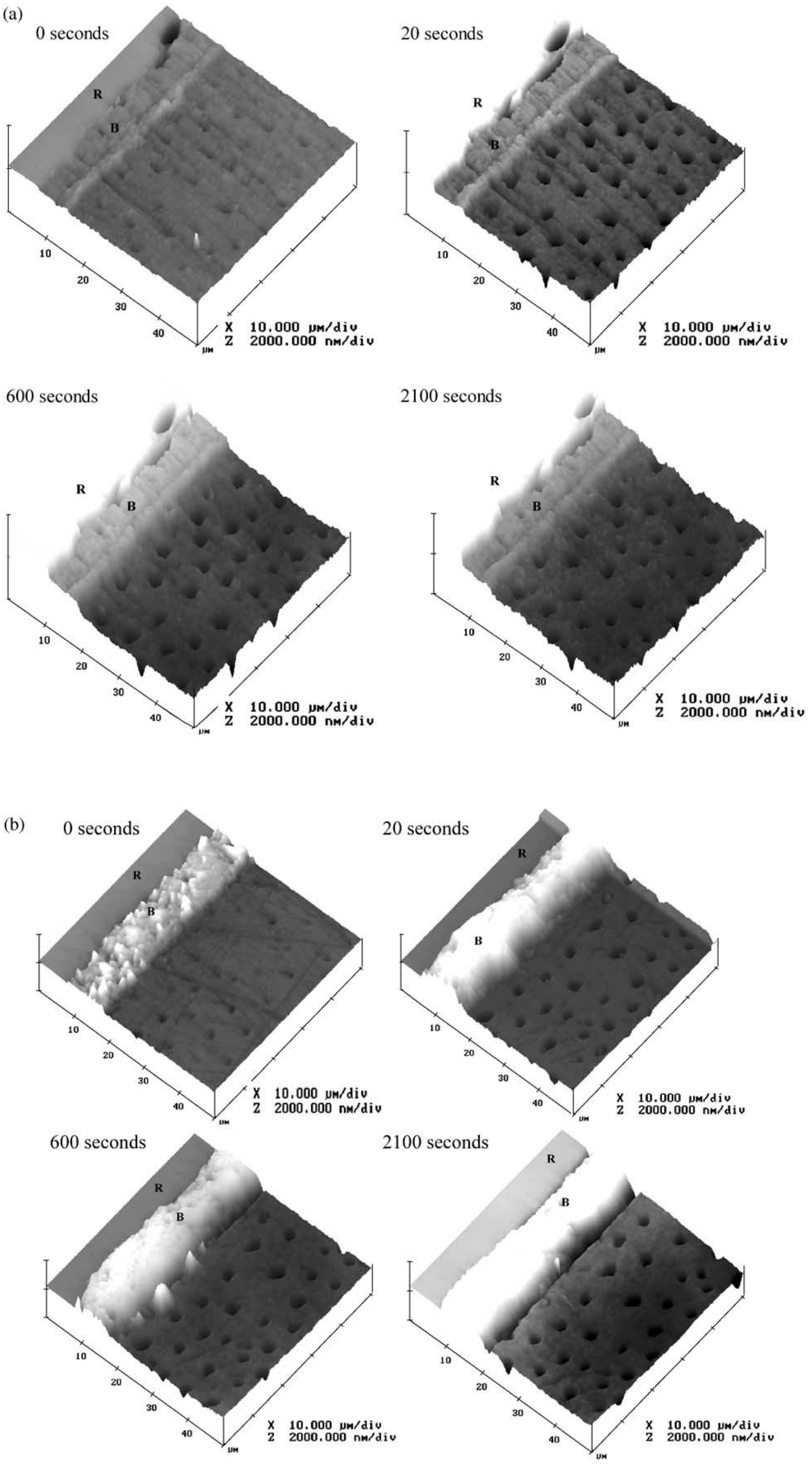
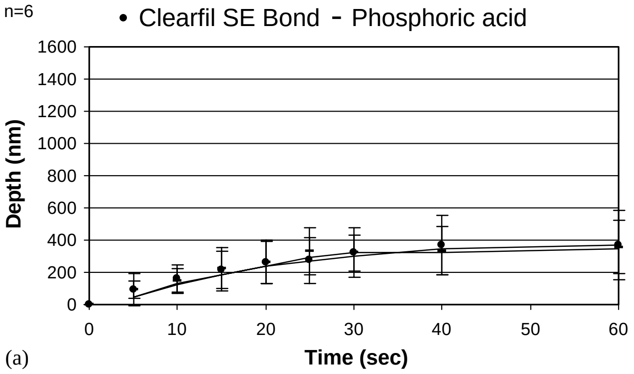


Fig. 2. AFM images of self-etching primer (a) and phosphoric acid (b) demineralization at different etching times. R is the glass reference layer and B is the bond layer between the glass and dentin.

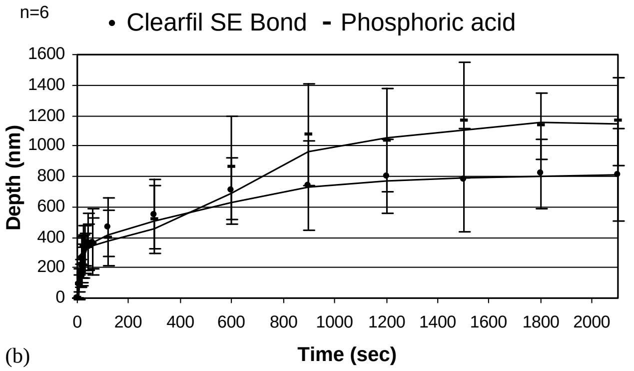
As seen in previous studies [14,17], intertubular dentin demineralization rates for both conditioners slowed after prolonged etching. In intertubular dentin there was no significant difference between conditioners in short-term etching rates over the first 20 s (13.0 ± 0.5 nm/s for the self-etching primer and 13.1 ± 0.5 nm/s for the phosphoric acid; $p = 0.92$; Fig. 3(a). A demineralization plateau was reached (i.e., the depth did not change more than 30% from one time point to another until the last measurement) at similar fractions of locations: 71% of the phosphoric acid specimen and 77% of the self-etching primer specimen ($p = 0.41$). However, among locations at which a plateau was reached, there was a significant difference between conditioners in the recession level at the plateau ($p < 0.05$; Fig. 3(b). For phosphoric acid the plateau was reached at 1100 ± 70 nm, whereas for the self-etching primer it was observed at 740 ± 70 nm.

Etching of peritubular dentin with either the primer or phosphoric acid widened the tubule lumens within 15 s (Fig. 2); they remained approximately the same dimension throughout the rest of the experiment. The peritubular dentin short-term etching rate with phosphoric acid, 40.0 ± 1.9 nm/s, was less than that of the self-etching primer, 67.9 ± 1.9 nm/s, by 28.2 ± 2.9 nm/s ($p < 0.001$) (Fig. 4).

The AFM measures changes in the dentin surface, and not in the demineralized front, so we used SEM micrographs (Fig. 5) to estimate the total dentin demineralization depth (2100 s) with the self-etching primer (Fig. 5(a)) and phosphoric acid (Fig. 5(b)). The SEM study of the demineralization depth is compared with the AFM study of surface recession, in Fig. 6. It can be seen that the depth of demineralization is much greater than the surface recession of the hydrated intertubular dentin determined by the AFM but the increased recession is related to total demineralization depth. Statistical analysis of the SEM data demonstrate low levels of correlation among measurements for the 20 s etching duration specimens and allowed image to be the unit of analysis, with a specimen size of 9 per conditioner. However in the 2100 s data, the high levels of correlation required disk to be the unit of analysis rather than measurement or image, with a specimen size of 3 per conditioner. The analysis revealed no statistically significant difference between the conditioners in demineralization depth after 20 or 2100 s etching. The mean (standard deviation) level was 2.3 (0.3) μm for the self-etching primer and 2.5 (0.3) μm for phosphoric acid after 20 s, for a difference of 0.21 (0.3) μm ($p = 0.15$). After 2100 s the mean (standard deviation) level was 10.8 (3.3) μm for the self-etching primer and 15.4 (2.2) μm for phosphoric acid, for a difference of 4.6 (2.8) μm ($p = 0.11$).



(a)



(b)

Fig. 3. Etching curves of intertubular dentin measured in AFM: (a) Curves for all samples at times up to 2100 s—A plateau was reached in the recession of the intertubular dentin for both conditioners at long-etching times, but for the self-etching primer it was at a shallower depth than for the phosphoric acid. (b) Initial recession curves from 0 to 60 s. The initial demineralization rates (initial 20 s) were not statistically different between conditioners ($p = 0.92$). The self-etching primer etching rate until 20 s was 13.0 ± 0.5 and 13.1 ± 0.5 nm/s for the phosphoric acid.

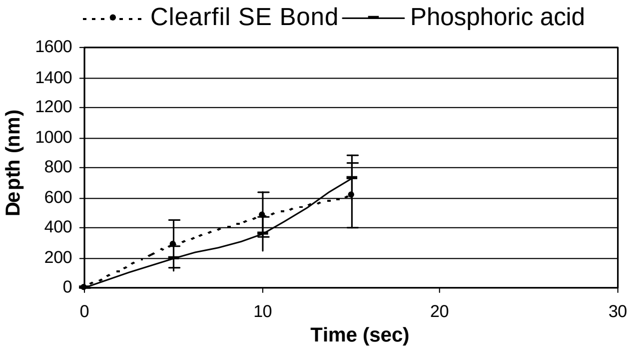


Fig. 4. Etching rate of peritubular dentin was measured only for the first 15 s of demineralization. The self-etching primer peritubular dentin etching rate (67.9 ± 1.9 nm) was significantly greater ($p < 0.001$) than the one observed with the phosphoric acid (40.0 ± 1.9 nm).

5. Discussion

The AFM has been successfully used in the past to study surface changes and the characteristics of dentin etched with a variety of acids [14–17,23]. In this study,

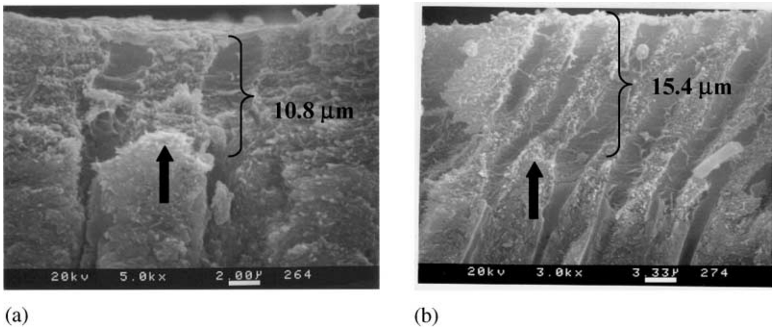


Fig. 5. SEM images of the cross-sectioned samples following demineralization: (a) 2100 s Clearfil SE Bond treatment gave widest demineralization of peritubular dentin; (b) 2100 s with 0.13% phosphoric acid gave a more longitudinal demineralization pattern. The differences may be related to conditioner viscosity. Demineralized front is marked with a block arrow. Micrographs have different magnifications to allow a better visualization of the demineralized layer. Measurements were performed accordingly.

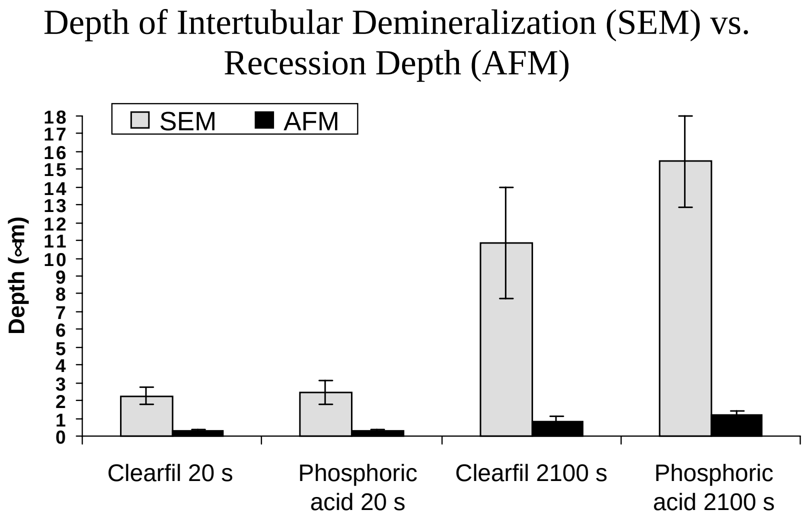


Fig. 6. Comparison of demineralization depths at 20 s and 2100 s for phosphoric acid and self-etching primer observed with SEM and recession depth with AFM. There was a significant difference in surface recession (AFM) between both conditioners at 2100 s and although not statistically different the demineralization depth caused by the phosphoric acid after 2100 s was 4.6 (2.8) µm deeper than the depth caused by the self-etching primer after the same time.

however, it was particularly challenging to execute the same type of measurements using a self-etching primer as a conditioner, since the self-etching primer has acidic monomers, which are not removed in clinical procedures and could interfere with the measurements. In preliminary experiments, the specimens were treated with a variety of solvents and examined with Fourier Transform Infra-red Spectroscopy (RFX-30 FTIR Spectrometer Laser Precision Analytical, Irvine, CA) to reveal the presence of monomer; ethanol and acetone proved to be efficient in removing the monomer. Ethanol was chosen since it has been reported to cause less dehydration of the dentin structure [24], and, as described earlier, the 5 min in deionized water totally reversed the shrinkage caused by the ethanol treatment.

Intertubular dentin etching followed the same pattern observed in previous studies with different diluted conditioners [14,17,25]. A plateau, where the depth changes stabilized, followed the initial demineralization in the majority of the specimens (Fig. 3(b)). This pattern of demineralization was attributed to the presence of water in the demineralized collagen matrix [14]. The initial faster recession could reflect the dissolution of the mineral closer to the surface and, as the demineralized front moves further away from the surface, the mineral loss is largely compensated by water. This increase in volume of water present in the demineralized structure could result in the dilution of the acid and progressively less demineralization.

The relation between AFM and SEM measurements (Fig. 6) suggests that the differences in depth at the apparent plateau of the recession versus time curves (Fig. 3(b)) are a result of the differences in total depth of demineralization. As the mineral is removed, the demineralized and hydrated collagen matrix retains most of its height and volume. However, continued demineralization will remove mechanical support of the collagen and the water cannot completely compensate for the loss of mineral. Therefore, the changes detected by the AFM measurements partially reflect the differences in total demineralization depth.

The more mineralized peritubular dentin had a higher etching rate than intertubular dentin, as previously observed [14,15]; this might be associated with the protective role of the organic matrix in dentin demineralization [26], which would retard the intertubular dentin demineralization, or the easier flow of the etchants through the tubule lumens giving faster contact with the peritubular dentin. With the phosphoric acid, intertubular dentin demineralization was greater, at long term, than with the primer, but at shorter etching times there was no significant difference in the amount of demineralization between the two conditioners. Although these short-etching times may be typical of clinical application times, they represent application of primer or acid in sequential 5 s increments, which is different than the clinical procedure.

The greater intertubular dentin demineralization caused by the phosphoric acid was detected by the AFM measurements after long etching times. Although the SEM data on the demineralization depth after 2100 s revealed a difference between conditioners of 4.6 (2.8) μm, this proved not to be statistically significant.

The intertubular surface recession caused by the phosphoric acid was significantly greater only after substantial demineralization, when the demineralized front was already quite deep, and could be related to the difference in viscosity between etchants. As a monomer, the primer has a higher viscosity, due to its higher molecular weight, than the aqueous solution of phosphoric acid. The lower viscosity of the phosphoric acid would allow somewhat easier diffusion through the demineralized dentin (porous media) and the difference would increase in proportion to the thickness of the demineralized dentin [27]. Thus, with increasing depth of demineralization, the phosphoric acid reaches the demineralized front faster than the primer, resulting in a significantly higher intertubular demineralization for the phosphoric acid, which was seen in the long-term data. Furthermore, due to its lower viscosity, the phosphoric acid could flow better through the tubules and demineralize the peritubular dentin in a vertical pattern, while the more viscous primer could be expected to flow less readily through the tubule lumens. Therefore, if the primer flows slowly down the tubules, we would expect a faster and more complete demineralization near the surface than at the subsurface area of the peritubular dentin. This horizontal pattern of demineralization could be better detected by the AFM tip, resulting in measurements that would show a statistically higher demineralization of the peritubular dentin with the primer. Thus, differences in demineralization between these etchants, with the same pH before application, could be due to their differences in viscosity, but this needs further investigation.

AFM measurements of dentin recession could be used additionally to evaluate the characteristics and kinetics of other self-etching primers and adhesives and compare them to a phosphoric acid with the same pH and viscosity. Furthermore, with the development, of a stable glass reference layer, suitable to analyze the demineralization effect of self-etching primers, it should be possible to evaluate the long-term effects of the self-etching primer acidity on the dentin. Although the self-etching primer demineralized the peritubular dentin more rapidly, our study shows that it gave the same level of intertubular demineralization as a phosphoric acid with the same initial pH, at cumulative times typical of those used in clinical practice.

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